Harris, Biophys. Soc. abstracts, 2012). To determine directly whether cMyBP-C binding affects tropomyosin position, we carried out 3D reconstruction of negatively stained thin filaments (containing F-actin, tropomyosin and troponin) decorated with the N-terminal fragment containing domains C0 to C2. Clear decoration was obtained under a variety of salt conditions. 3D reconstructions suggest that under most conditions cMyBP-C does not displace tropomyosin from its low Ca^{2+} position, although under certain conditions some shift of tropomyosin did appear to occur. At high Ca^{2+}, there was little effect on tropomyosin position. The results suggest that cMyBP-C may modulate thin filament function by physically competing with tropomyosin for its low Ca^{2+} site on F-actin.

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Calcium-Calmodulin Competes with Actin for Binding to the M-Domain of Cardiac Myosin Binding Protein-C
Samantha P. Harris, Elaine Hoye.
University of California, Davis, Davis, CA, USA.
The regulatory M-domain of cardiac myosin binding protein-C (cMyBP-C) binds to myosin, actin, and to calmodulin when calcium is present (i.e., calcium-calmodulin), but it is unclear whether binding of all three ligands is independent or if binding interactions are competitive. Here we investigated whether calcium-calmodulin (Ca-Cam) binding to the M-domain affected the ability of the M-domain to bind to actin using cosedimentation binding and calmodulin-sepaharose pull-down assays. Results of actin cosedimentation binding assays showed that Ca-Cam significantly reduced specific binding of a recombinant protein containing three N-terminal domains of cMyBP-C (i.e., C1-M-C2, referred to as C1C2) when 10 μM calmodulin was present in the presence of calcium (pCa 3.0). In the absence of calcium (at pCa 10.0) calmodulin had no effect on C1C2 binding to actin. Increasing Ca-Cam concentrations to achieve higher molar ratios with respect to C1C2 further reduced the amount of C1C2 that bound to actin. Conversely, in calmodulin-sepharose pull-down experiments, binding of C1C2 to calmodulin was only modestly reduced in the presence of increasing concentrations of F-actin. Taken together, these results indicate that binding of Ca-Cam can compete with actin for binding to the M-domain. These results suggest a potential mechanism whereby the functional effects of cMyBP-C binding to actin can be regulated by calcium. This work supported by NIH HL080367.

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Structural and Functional Studies of Phospholamban-Sarcolipin Chimeras
Przemek Gorski, Joseph Primeau, John Paul Glaves, **Howard S. Young**.
University of Alberta, Edmonton, AB, Canada.
The sarcoplasmic reticulum (SR) is a calcium storage organelle in muscle cells that contains a calcium pump (SERCA) required for calcium reuptake and muscle relaxation. The activity of SERCA is regulated by the small integral-membrane subunits phospholamban (PLB) and sarcolipin (SLN). PLB is present in cardiac and smooth muscle, while SLN is found in skeletal muscle and the atria of the heart. Consequently, the regulatory mechanisms imposed by PLB and SLN have clinical implications for the treatment of heart disease. There is significant sequence homology in the transmembrane regions of PLB and SLN suggesting a similar mode of binding to SERCA; however, SLN has a unique and highly conserved C-terminal tail (27RSYQY) that is lacking in PLB. The structural differences in the luminal domains between these two proteins could be responsible for the subtle differences in their regulation of SERCA. We have functionally characterized alanine mutants of the C-terminal tail of SLN using co-reconstituted proteoliposomes that mimic the SR membrane. We found that Arg27 and Tyr31 are essential for SLN function. To further study the role of the luminal tail of SLN we also tested the effect of a truncated variant of SLN (Arg27stop) as well as chimeras of PLB consisting of the wild-type sequence with the five luminal residues of SLN added to its C-terminus. The Arg27stop form of SLN resulted in loss of function, while the PLBtail chimeras resulted in super-inhibition with characteristics reminiscent of the SERCA-PLB-SLN ternary complex. These functional results are being correlated with structural studies by cryo-electron microscopy of SERCA in complex with the PLBtail chimeras. Based on our results, we propose that SERCA inhibition by SLN is encoded in the C-terminal tail, and that the functional properties of SLN are transferred to PLB in the PLBtail chimeras.

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Characterization of Cardiac Adiponectin Post-Translational Processing and Secretion
Amanda Manly, Stephanie Kern, Joanna Solarewicz, Naama Sleiman, Stephanie Lucas, Todd Leff, Steven Cala.
Wayne State University, Detroit, MI, USA.
Adiponectin (ADN) is primarily secreted from adipocytes, and serum concentrations negatively correlate with obesity, diabetes, and several other metabolic diseases. More recently, secretion and autocrine actions of cardiac ADN have been described. The purpose of this study was to characterize the post-translational modifications undergone by ADN monomers in cardiac and other mammalian, and their association into trimers, hexamers, and larger multimers (>18-mers) during secretory pathway transport. Adiponectin-flag adenovirus (Ad.ADNf) was used to overexpress the protein in mammalian cells and primary adult rat cardiomyocytes. Intracellular and secreted adiponectin were analyzed in cell extracts and 24 h supernatants. Native oligomeric adiponectin within these cellular systems was analyzed by immunofluorescence, while composite monomers and multimers were examined by SDS-PAGE and immunoblotting. Intracellular cardiomyocyte ADN accumulated in junctional SR puncta, an early ER subcompartment, consistent with current views of the cardiac secretory pathway, whereas it co-localized with classical ER markers in HEK and COS cells. Cardiac ADN was secreted as a collection of polymers comprised of a single relatively unmodified protein monomer, similar to that secreted from cultured epididymal fat. Roughly half of the protein was secreted in 24 h. In contrast, ADN from HEK cells secreted polymers comprised of ADN monomers with slightly greater modification. Minor changes in response to treatments with tunicamycin suggest that some portion of both the intracellular and secreted protein monomers undergo N-linked glycosylation. HEK cells alone exhibited significant ability to cleave ADN after its secretion. Among all mammalian cells, both intracellular and secreted ADN were comprised of distinct patterns of protein polymers. Similarities between monomeric and polymeric structures in secreted ADN and effects on adenosine monophosphate-kinase from different mammalian cells support the possibility that ADN serves both endocrine and autocrine actions for fat and heart, respectively.

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Na/K ATPase Affects Respiration Kinetics and Provides Evidence for Intracellular Diffusion Restrictions in Permeabilized Rat Cardiomyocyes
Mervi Sepp, Svetlana Kotljarova, Marko Vendelin.
Institute of Cybernetics, Tallinn University of Technology, Tallinn, Estonia.
Restrictions in the intracellular movement of adenosine nucleotides play an important role in cardiac energetics. In the work leading up to this project we confirmed that strong functional coupling exists between some ATPases and mitochondria. Such coupling is evidence of restricted diffusion in the cell. In addition our results revealed a previously unknown feedback mechanism showing that endogenous PK and a fraction of ATPases are tightly coupled. The localization of the diffusion restrictions grouping ATPases with ATP production and to what cellular structures they can be attributed to remains unknown. To address this issue we inhibited cellular ATPases one by one and analyzed their impact on respiration kinetics. The aim of this work is to investigate if sarcolemma Na/K ATPase (NKA) is or is not coupled to endogeneous PK.
We found that NKA affects cellular energetics inducing a 45% drop in ATPase activity initiated by 2mM ATP. To investigate the effect further we performed a set of six experiments used on the control cells to compare the kinetic parameters. We found that for NKA inhibited cells Km(ADP) was significantly higher and Km(ATP) significantly lower than in the control case. Vmax for both ADP and ATP were lower for NKA inhibited cells than in the control case. We also saw a different response in respiration to PEP indicating a change in coupling between ATPases and endogeneous PK. We then analyzed this data using seven mathematical models with different compartmental specifications. This work presents the experimental results and our analysis of this data using these models.

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Proteomic Analysis of Akita Mice Reveals 9 Proteins Altered during Early Stages of Diabetic Cardiomyopathy
Shannamar Dewey, Mandeep Sohal, Aldrin V. Gomes.
UC Davis, Davis, CA, USA.
Cardiovascular disease is the leading cause of diabetic morbidity and over 10% of patients with type 1 diabetes (T1DM) die before they are 40 years old. This study utilized Akita mice, a murine model with T1DM progression analogous to that of humans. Diabetic cardiomyopathy in Akita mice presents as diastolic impairment as early as 3 months of age and significant cardiac atrophy by 5 weeks. Hearts from recently diabetic mice (5 weeks) were analyzed with label free proteomics to identify proteins which may play a critical role in the pathophysiology of diabetic cardiomyopathy (n=3). At this early stage, 9 proteins were differentially expressed in diabetic mice: GANC, PLEKHN1, COLIA1, GSTK1, ATP1A3, RAP1A, ACADS, EEF1A1, HRC, EPHX2, and PKP2 (gene names). A recent study demonstrated that deletion of EPHX2,